thesis of short oligomers of deoxynucleosides linked by pyrophosphate bonds is, therefore, easier than the formation of equivalent oligonucleotides. Furthermore, template-directed reactions are, as we have shown, much less dependent on the choice of the activated substrate and the reaction conditions. Even the very simple and potentially prebiotic acyclonucleoside derivative ImpĞpIm (*1*) was an adequate substrate.

In other experiments we have shown that poly(C) will direct the efficient copolymerization of 2-MeImpG and ImpdGpIm (*1*) at all ratios of the two substrates (*10*). Thus it seems plausible that a poly(C) template could bring about a copolymerization of components selected from a complex mixture of partially and fully phosphorylated G derivatives. If, as we expect, a poly(C) analogue in which pyrophosphate groups have partially or fully replaced the normal phosphate groups will itself act as a template for the condensation of G derivatives, it will indicate that template-directed synthesis is much less demanding than has been demonstrated previously.

Our results also suggest, but certainly do not prove, that a great variety of template-directed reactions may be possible with unconventional substrates. Substrates **4** and **5**, for example, in the presence of poly(C), might react to form amide-linked polymers when the phosphate is activated in **4** (*11*) or the carboxyl in **5**, while substrate **6** might form disulfide-linked oligomers on oxidation (Fig. 1). While such reactions may not be interesting in the context of the origins of life on the earth, they would greatly extend the scope of template-directed organic synthesis.

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1. Abbreviatons: A, adenosine; C, cytidine; G, guanosine; U, uridine; Ğ, 9-(1,3-dihydroxy-2-propoxy)methylguanine; dG, 2′-deoxyguanosine; pN (N is A, G, or dG), the 5′-phosphate of N; 2-MeImpN, the 2-methylimidazolide of pN; Np, the 3′-phosphate of N; ImpNpIm, the 3′,5′-diphosphoimidazolide of N; ImpĞpIm, the 1,3-diphosphoimidazolide of Ğ (compound **3**).
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Epizootic Carcinoma in the Winter Flounder, *Pseudopleuronectes americanus*

Abstract. *The winter flounder,* Pseudopleuronectes americanus, *is an esteemed food fish and has sustained an important commercial and recreational fishery for many years in the northeastern United States. Histopathologic examinations of hepatic tissues of winter flounder from Boston Harbor revealed a high prevalence of neoplasms. The lesions, designated as cholangiocarcinomas and hepatocarcinomas, were found in 16 of 200 fish examined and resembled those experimentally induced in rodents by exposure to carcinogens.*

Neoplasms have been noted in many marine fish species, and they affect virtually all organs (*1–3*). Most of the tumors were described incidental to the conduct of other studies and usually occurred in a single animal. Recently, epizootic neoplasia was noted in localized populations of several bottom-dwelling marine fishes from the U.S. East and West coasts. Hepatomas (adenomas, hepatocarcinomas, and cholangiocarcinomas) have been found in English sole (*Parophrys vetulus*) from the Duwamish River, a tributary of Puget Sound (*4, 5*), and in tomcod (*Microgadus tomcod*) from the Hudson River estuary (hepatocarcinomas) (*6*). The only other epizootic neoplasm in a marine fish in the United States is the neurilemoma of the bicolor damselfish (*Eupomacentrus partitus*) from the Florida Keys (*7*). Although specific carcinogens [polycyclic aromatic hydrocarbons (PAH's) and polychlorinated biphenyls (PCB's)] have been suggested as the inducers of the hepatomas of English sole and tomcod, it is unlikely

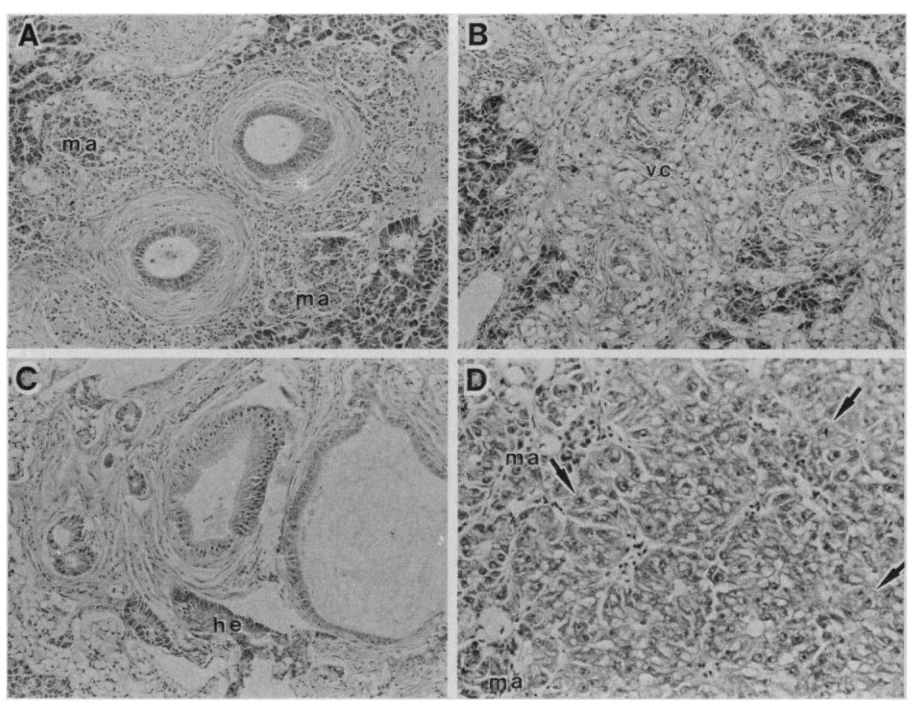

Fig. 1. (A) Pericholangitis. Leukocytes and macrophage aggregates (ma) surround atypical bile ducts (×20). (B) Vacuolated cells. Vacuolated cells (vc) form acini and are enveloped by fibrous tissue (×20). (C) Bile duct dilation and hyperplasia. Hyperplastic epithelium (he) is contiguous with vacuolated cells (×20). (D) Adenoma. Macrophage aggregates (ma) define outer edge of neoplasm. Note mitoses (arrows) (×40). Stain, hematoxylin and eosin.

Table 1. Distribution of presumed preneoplastic and neoplastic hepatic lesions in winter flounder from Boston Harbor. Of 200 fish examined, 20 had preneoplastic or neoplastic lesions.

Focus (n = 16)		Adenoma	Cholangioma*	Carcinoma† (n = 15)	
Basophilic	Vacuolar			Biliary	Hepatocellular
7	9	1	1	14	5

*Of 15 fish with carcinomas, 1 also had a cholangioma. †Of 15 fish with carcinomas, 4 had both cholangiocarcinomas and hepatocarcinomas and 12 had basophilic (3) or vacuolar (9) foci; 1 fish had both basophilic and vacuolar foci.

that the neurilemoma of the bicolor damselfish is induced by an environmental carcinogen.

Specific integumental lesions and skeletal and pigmentation anomalies have been used as markers to define coastal areas of compromised fish health and environmental quality on the Middle Atlantic and Northeast coasts since 1979. A recent evaluation of 6 years of data (*8*) showed that the distribution of these diseases is discontinuous. For most of the diseases enumerated, prevalences are higher in coastal waters adjacent to large population centers; however, only one flatfish species, the winter flounder (*Pseudopleuronectes americanus*), is distributed throughout the entire area sampled and only one disease, fin erosion, shows a consistent pattern of increased prevalence in areas contiguous to urban development. Although the specific causes of fin erosion are not known, the disease is found in both cultured and wild fish under conditions of compromised environmental quality. Because of the higher prevalence of fin erosion in winter flounder from nearshore environments, we undertook an intensive examination of flounder from selected nearshore areas between Maine and Maryland in order to determine whether other aspects of their health were affected.

Gross and microscopic examinations of liver tissues excised from winter flounder and another resident flatfish, windowpane flounder (*Scophthalmus aquosus*), from Connecticut (New Haven Harbor) and Rhode Island (upper Narragansett Bay) between October and December 1983 disclosed a variety of microscopic lesions. A total of 119 trawl-caught flatfish were examined (36 from New Haven Harbor and 83 from Narragansett Bay); 61 were winter flounder (total length, 18 to 44 cm) and 58 were windowpane flounder (21 to 32 cm). None of the fish had gross hepatic lesions. Nonneoplastic lesions included focal hepatitis and necrosis, biliary hyperplasia, cholangiofibrosis, and hypertrophy and hyperplasia of macrophage aggregates (*9*). Several winter flounder had focal and diffuse areas of vacuolated cells that frequently appeared "organized" and acinar in configuration. Hepatic lesions presumed to be preneoplastic on the basis of criteria proposed for the nomenclature of proliferative hepatocellular lesions in rodents (*10, 11*) included eosinophilic, basophilic, and vacuolar cell foci. Neoplastic lesions included adenomas, hemangiomas, hepatocarcinomas, and cholangiocarcinomas. Overall, 12 of 119 (10 percent) of the winter and windowpane flounder from Connecticut and Rhode Island had preneoplastic and 4 of 119 (3.4 percent) had neoplastic hepatic lesions. Lesions were found only in fish over 25 cm long.

The high prevalence of preneoplastic and neoplastic lesions in these fish dictated continued sampling through the winter and expansion of the sampling to other East Coast areas. Winter flounder from certain areas in Boston Harbor have a high prevalence of fin erosion (*12*). This observation, together with the fact that examination of flatfish from Boston Harbor would increase the number of coastal locations sampled, prompted us to ask the Massachusetts Division of Marine Fisheries for winter flounder from the harbor. On 2 April and 26 June 1984, 100 trawl-caught fish were obtained from Boston Harbor; the 200 fish ranged in length from 22 to 42 cm (mean, 35.3 cm). Only flounder collected from the southern shore of Deer Island had grossly visible hepatic lesions. Nonneoplastic lesions in these fish included pericholangitis, vasculitis, focal and single-cell parenchymal necrosis, biliary hyperplasia, cholangiofibrosis, and hypertrophy and hyperplasia of macrophage aggregates (Fig. 1). Many bile ducts were substantially dilated and had short columnar, cuboidal, and in some instances squamous epithelium. Most prevalent were hypertrophy and hyperplasia of macrophage aggregates (68 percent) and vacuolar cell lesions (also 68 percent).

The vacuolated cells contained eccentric, small, compact nuclei in a clear cytoplasm and occurred as focal or diffuse lesions. In many sections these cells formed acinar and tubular patterns resembling ductal cross sections; however, cell nuclei were invariably luminal. In some sections the acinar formations had a periodic acid Schiff (PAS)–positive lu-

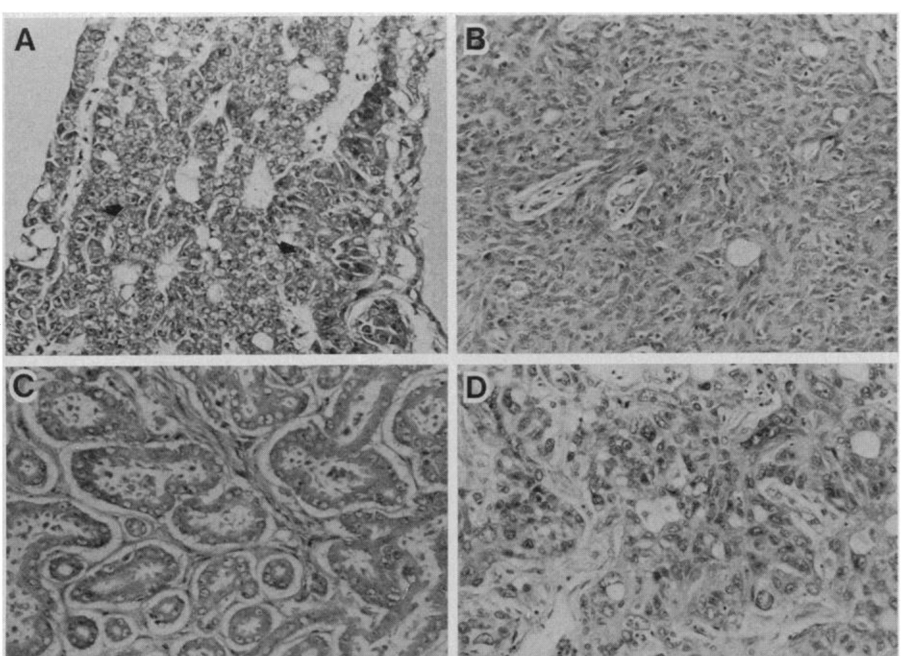

Fig. 2. (A) Well-differentiated hepatocarcinoma. Hepatocytes have lost polarity and contain numerous eosinophilic, hyaline, cytoplasmic inclusions (arrows) (×40). (B) Anaplastic hepatocarcinoma. Hepatocytes are spindle-shaped, giving the tumor the appearance of a fibrosarcoma (×40). (C) Well-differentiated cholangiocarcinoma. Some of the variants of this lesion are fibrous, more cellular, and have intraluminal aggregates of desquamated cells (×40). (D) Anaplastic cholangiocellular carcinoma. Nuclei vary considerably in size and shape (×40). Stain, hematoxylin and eosin.

minal border. Often, the acini of vacuolated cell foci were enveloped by connective tissue; in other cases the vacuolated cells were apparently continuous with hyperplastic biliary epithelium, which also was vacuolated. Mitoses were frequently seen in normal and hyperplastic ductal epithelium as well as in foci containing the vacuolated cells. Frozen sections of Formalin-fixed tissues stained with Oil Red O and Sudan B were negative. Specific stains to demonstrate glycogen (PAS) and mucin (Alcian Blue) also were negative. The origin of these vacuolated cells is unknown; however, all flounder with neoplasms also had vacuolated cell lesions. Large vacuolated cell foci beneath the hepatic capsule bulged and slightly compressed adjacent tissue.

In several of the large vacuolated cell foci morphologically different cells were present marginally or centrally. In some sections these cells had a finely granular, basophilic cytoplasm and large vesicular nuclei. Cytologically, they resembled ductal epithelial cells. Cell arrangement was acinar, and mitoses were present. Other sections showed focal areas of polygonal cells with deeply basophilic cytoplasm and mitoses. These cells resembled hepatocytes more than ductal cells; however, their arrangement also was acinar.

Lesions identified as preneoplastic or neoplastic (Table 1) were noted in 10.0 percent of the fish sampled from Boston Harbor; however, the actual percentage of fish with these lesions may have been considerably higher since serial "skip" sections of several randomly selected livers revealed neoplastic foci in fixed tissues with no apparent lesion in the initial section. Basophilic foci were present as irregularly shaped areas of enlarged hyperchromatic hepatocytes. Examination at higher magnification usually revealed prominent, hypertrophic nuclei and nucleoli. The hepatocellular adenoma contained cells that were more basophilic than adjacent parenchymal cells. It could be readily recognized by its sharp demarcation from adjacent tissue and a perimeter of mitotic cells. The neoplasm compressed adjacent parenchymal cells. Macrophage aggregates were present in parenchyma adjacent to the lesion but not in its interior. Hepatic cords did not differ substantially from those of adjacent parenchyma; however, bile ducts were not present.

Neoplasms designated as cholangiocarcinomas and hepatocarcinomas (7.5 percent) differed in different fish livers as well as in different sections from a single liver. Poorly differentiated hepatocarcinomas consisted of anaplastic, basophilic, spindle-shaped cells. The lesion had a solid appearance and resembled a fibrosarcoma; however, trichrome stains revealed the cell of origin to be parenchymal and not stromal; few mitoses were present. The edge of the lesion was not sharply demarcated from adjacent parenchymal cells. A second type of hepatocarcinoma consisted of solid, sometimes acinar, formations of smaller, polygonal, intensely basophilic hepatocytes. Some of the hepatocytes contained spherical, eosinophilic, cytoplasmic inclusions. In these neoplasms hepatic cordal architecture was atypical. Cavities were sometimes present in these lesions and contained amorphous eosinophilic material, leukocytes, and macrophages.

Another hepatic neoplasm was not unequivocally identifiable. The differentiation of cholangiocarcinomas from hepatocarcinomas is difficult because of the epithelial origin of both types of lesions. Although ductal hyperplasia and cholangiofibrosis were evident in many sections, some sections contained a proliferative lesion that resembled cholangiocarcinoma. The neoplasm consisted of epithelial-like cells with large vesicular nuclei. Ducts at the perimeter of the lesion were smaller and more regular in size than those in the interior. Many ducts contained effete epithelial cells and an amorphous eosinophilic material. The epithelial-like cells exhibited numerous "blebs" at the luminal border. Many cells at the edge of the lesion were mitotic. Although the appearance and ductal configuration of the constituent cells indicated a cholangiocarcinoma, the neoplasm could have been a hepatic adenocarcinoma. Mixed carcinomas (within the same lesion) were not seen.

Compared with livers of winter flounder obtained from other areas, the Boston Harbor fish livers are unique. Although focal and diffuse areas of necrosis, inflammation, and vacuolated cells have been seen in livers of winter flounder from other Northeast estuaries (Connecticut, Rhode Island), the lesions were not so abundant or severe or as strongly associated with extensive neoplasia. None of 93 winter flounder (total length, 13 to 58 cm; mean, 30.0 cm) collected from unpolluted sites on the south shore of central and eastern Long Island, New York; Casco Bay, Maine; and Georges Bank had neoplastic lesions. The high prevalences of macrophage aggregate hyperplasia and vacuolated cell lesions in Boston Harbor flounder are consistent with the action of a hepatotoxin. The hepatic architecture of teleost fish, unlike that in homeotherms, lacks a distinctly lobular configuration. Visualization of portal triads around a central vein is infrequent. In winter flounder livers with multiple microscopic lesions, the initial site of action of a hepatotoxin is indeterminable and is certainly not discernible as portal or centrolobular.

One can speculate that vacuolated cells represent the primary toxin-induced lesion. Organizationally and cytochemically, these cells seem more like ductal epithelial cells than hepatic parenchymal cells; however, this is equivocal. There was no indication in the tissues examined that the vacuolated cell lesions are reversible; on the contrary, some vacuolated cell foci were necrotic, infiltrated by macrophages and other leukocytes, and invested with fibrous tissue. The most significant change in the behavior of these cells may involve another pathway. Surviving cells may be committed to neoplasia, with the transformed cells ultimately forming carcinomas. The sections examined present a diverse spectrum of proliferative morphological changes (Fig. 2) that are comparable to those experimentally induced with carcinogens in rodents; however, additional studies are required to establish specific cause-and-effect relations.

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